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Effect of Clinical Phenotype on Yield of Long QT Syndrome Genetic Testing

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OBJECTIVES The purpose of this study was to examine the effect of clinical phenotype on the yield of genetic testing for congenital long QT syndrome (LQTS).

BACKGROUND Since the discovery of the first LQTS susceptibility genes in 1995, numerous genotype-phenotype relationships have emerged during the past decade of research genetic testing. In May 2004, LQTS genetic testing became a clinically available molecular diagnostic test.

METHODS Blinded to genetic test results, analysis of the clinical phenotype was performed in 541 consecutive unrelated patients referred to Mayo Clinic's Sudden Death Genomics Laboratory for LQTS genetic testing from August 1997 to July 2004.

RESULTS The yield of genetic testing correlated significantly with the corrected QT interval (QTc) and clinical diagnostic score ranging from 0% when QTc was <400 ms to 62% when QTc was >480 ms ($p < 0.0001$). Among those with the highest clinical probability, the yield was 72% (89 of 123). The yield fluctuated substantially depending on age at diagnosis in males. Among physicians who referred ≥5 patients, the yield ranged from 0% to 80% ($p < 0.0001$).

CONCLUSIONS In this large cohort of unrelated patients referred for LQTS genetic testing, the clinical phenotype strongly correlated with the likelihood of elucidating a pathogenic mutation with the cardiac channel gene screen. (J Am Coll Cardiol 2006;47:764–8) © 2006 by the American College of Cardiology Foundation

Affecting 1 in 5,000 persons, long QT syndrome (LQTS) is the prototypic cardiac channelopathy underscored by profound genetic and phenotypic heterogeneity (1,2). To date, over 400 mutations in five cardiac channel-encoding genes have been identified: LQT1 (*KCNQ1*-encoded potassium channel [I_{Ks}] mutations), LQT2 (*KCNH2*-encoded potassium channel [I_{Kr}] mutations), LQT3 (*SCN5A*-encoded sodium channel mutations), and LQT5 and LQT6 (*KCNE1*- or *KCNE2*-encoded potassium channel beta subunit mutations) (3–6). The LQT4 stems from ankyrin-B mutations and represents the first nonchannel form of LQTS (7).

Over the past decade, genetic testing for LQTS, particularly the three most common genotypes of LQT1 to LQT3, have revealed relatively gene-specific electrocardiographic profiles, responses to epinephrine, arrhythmogenic triggers and arrhythmogenic temporal states, responsiveness to beta blockers, treatments, and prognosis (8,9). In May 2004, genetic testing for the five LQTS-associated channel genotypes became a commercially available clinical diagnostic test in the U.S. (10).

Clinically, genetic testing has been used to risk-stratify patients, guide treatment decisions, and precisely elucidate the "carrier" status of potential at-risk relatives (11–14). Having completed comprehensive mutational analysis in one of the largest assembled cohorts of unrelated LQTS referrals (6), we scrutinized the effect of clinical phenotype on the yield of LQTS genetic testing.

METHODS

Study population. Between August 1997 and July 2004, 541 consecutive unrelated patients were referred by 103 physicians to Mayo Clinic's Sudden Death Genomics Laboratory for LQTS genetic testing in accordance with IRB-approved research protocols. Previously, an LQTS-associated channel genotype was established in 272 of 541 cases (6,15,16).

Blinded to genotype, clinical phenotype including ethnicity, sex, age at diagnosis, presence or absence of syncope, seizures, or aborted cardiac arrest, temporally related triggers, family history, and 12-lead electrocardiogram (ECG) was recorded. A cumulative LQTS diagnostic "Schwartz and Moss" score (which is derived in part from the corrected QT interval [QTc], symptoms, and family history) was assigned (17). A cumulative score of ≥4 suggests a robust phenotype and strong probability for LQTS. Sufficient information to derive a clinical score was available in 417 of 541 cases (77%). The QTc data were available for all 417

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Abbreviations and Acronyms
LQTS = long QT syndrome
QTc = corrected QT interval

cases, and an ECG for review and independent calculation was available for 341 cases (63%).

Differences between continuous variables were evaluated using unpaired Student *t* tests, and nominal variables were analyzed using chi-square analysis. The chi-square test was used for multiple group comparisons. Statistical significance was considered at $p < 0.05$.

RESULTS

Table 1 summarizes the clinical phenotype. Figure 1 details the distribution of genotypes: LQT1 (n = 120), LQT2 (n = 93), LQT3 (n = 26), LQT5 (n = 3), LQT6 (n = 1), and multiples (n = 29). Nearly one-half of the cohort (269 of 541) had no LQTS-associated channel mutation and are designated "genotype negative."

The genotype positive subset (n = 272) had a significantly distinct clinical phenotype compared with the genotype negative subset (n = 269) in terms of QTc (494 ± 51 ms vs. 470 ± 60 ms; $p < 0.0001$) and cumulative LQTS diagnostic score (Table 1). Forty-one percent of the genotype positive subset had a clinical score of ≥4 compared with 17% of the genotype negative subset ($p < 0.0001$). There was no difference in sex, ethnicity, age at diagnosis, or history of cardiac arrest between the two subsets. Presence of syncope and family history both trended toward an increase among genotype-positive individuals (46%) compared to genotype-negative individuals (38%, p = 0.067).

The yield among white subjects (n = 483) was 49% compared with 13 of 19 (68%) Hispanics, 8 of 11 blacks (73%), 2 of 3 Asians, 1 of 1 Native American, and 12 of 24 where the ethnicity was not defined. Although the yield was greater among nonwhites than whites (24 of 34 [71%] vs. 236 of 483 [49%]; $p < 0.02$), ethnicity was not an independent predictor. Rather, the QTc and clinical scores were greater among this small subset of nonwhites (data not shown). Among those with a positive family history of LQTS-attributable symptoms or premature sudden death, the yield was 55%.

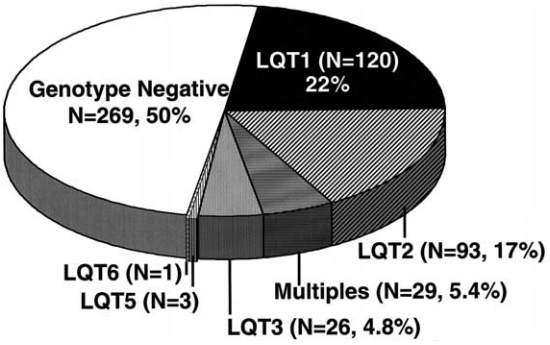

Figure 1. Summary of long QT syndrome genotypes among 541 consecutive unrelated patients.

Figure 2 depicts the age and gender distribution with an average age at diagnosis of 24 ± 16 years, ranging from 1 day to 78 years (Table 1). Over two-thirds of the patients were under 30 years at diagnosis and males were significantly younger than females (18 ± 16 years vs. 25 ± 15 years; $p < 0.0001$). Overall, gender had no effect on the yield, with 178 of 358 (50%) females and 94 of 183 males (51%) (p = 0.28) being genotype positive.

However, the yield fluctuated substantially depending on age at diagnosis in males but not in women: 25 of 30 (83%) males diagnosed at ≤5 years of age were genotype positive compared with only 13 of 39 (33%) males diagnosed

Table 1. Demographics of 541 Consecutive Unrelated Patients Referred for Long QT Syndrome Genetic Testing: Comparison of Genotype-Positive and Genotype-Negative Subsets

	Total Cohort	Genotype-Positive	Genotype-Negative	p Value
Number of unrelated patients	541	272	269	NS
Age at diagnosis (yrs) (range)	24 ± 16 (0–78)	23 ± 16 (0–75)	25 ± 16 (0–78)	NS
Gender (male/female)	183/358	94/178	89/180	NS
Ethnicity (% white)	93	90	96	NS
Average QTc (ms) (range)	482 ± 57 (365–759)	494 ± 51 (402–700)	470 ± 60 (365–759)	<0.0001
% with QTc >480 ms	46 158/341	57 97/169	35 61/172	<0.0001 3.3×10^{-7}
% with syncope	42 227/541	46 126/272	38 101/269	0.067
% with cardiac arrest	12 68/541	13 36/272	12 32/269	NS
% with positive family history	42 228/541	46 126/272	38 102/269	0.067
% with "Schwartz and Moss" score ≥4	29 123/417	41 89/218	17 34/199	<0.0001 1×10^{-9}

In addition to percentages, absolute numbers for the various clinical parameters are provided as well.
QTc = corrected QT interval.

Figure 2. Age and gender distribution.

between 11 and 15 years of age ($p < 0.0001$) (Fig. 3). During the first five years of life, the yield was greater in males (83%) than in females (53%) ($p = 0.01$). Closer inspection shows that this difference was due to the yield in the subset of 28 patients diagnosed during the first year, wherein 11 of 14 (79%) males were genotype positive compared to 4 of 14 (29%) females ($p = 0.02$). Unlike ethnicity, age and male gender appear to independently impact the yield of genetic testing, because the QTc and clinical scores were similar between males and females at the various age categories (data not shown).

The yield correlated significantly with QTc and clinical ("Schwartz and Moss") score (Fig. 4). The yield ranged from 0% for the 7 subjects referred for LQTS genetic testing despite a resting QTc of <400 ms to 62% for the 144 subjects with a QTc of >480 ms ($p < 0.0001$) (Fig. 4A). Among the 417 unrelated cases with an assigned clinical score, an LQTS genotype was established for 43% of subjects (93/215) when the clinical score was <4. The yield was 44% among the 124 patients with insufficient clinical data to assign a score. In contrast, among those with the highest clinical probability for LQTS (score ≥4), 89 of 123 (72%) were genotype positive: LQT1 (n = 39), LQT2 (n = 30), LQT3 (n = 5), LQT5 (n = 1), and multiple (n = 14) ($p < 0.0001$) (Fig. 4B).

Finally, we examined the effect of referral source (103 referring physicians and 91 direct patient self-referrals) on the yield. Among the 18 physicians who referred ≥5 patients (290 total), the average yield was 51%, which was no different than the 85 physicians who referred <5 patients (49%) or the self-referrals (49%). However, the yield varied widely among these 18 physicians, ranging from 0% to 80% ($p < 0.0001$). Within our LQTS clinic, there were 22 unrelated patients assigned a clinical score of ≥4 following their comprehensive and systematic assessment by the same physician. Subsequently, an LQTS-associated mutation was elucidated in all 22 (100% yield).

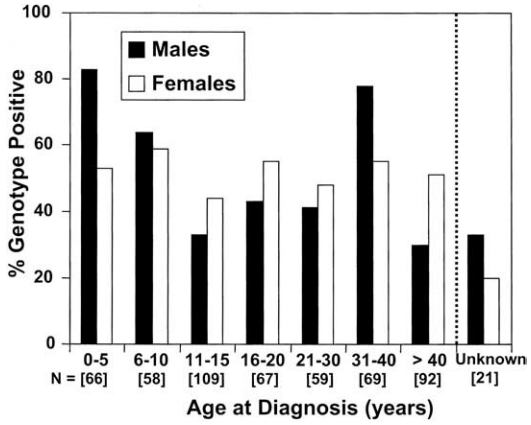

Figure 3. Effect of age and gender on yield of long QT syndrome genetic test.

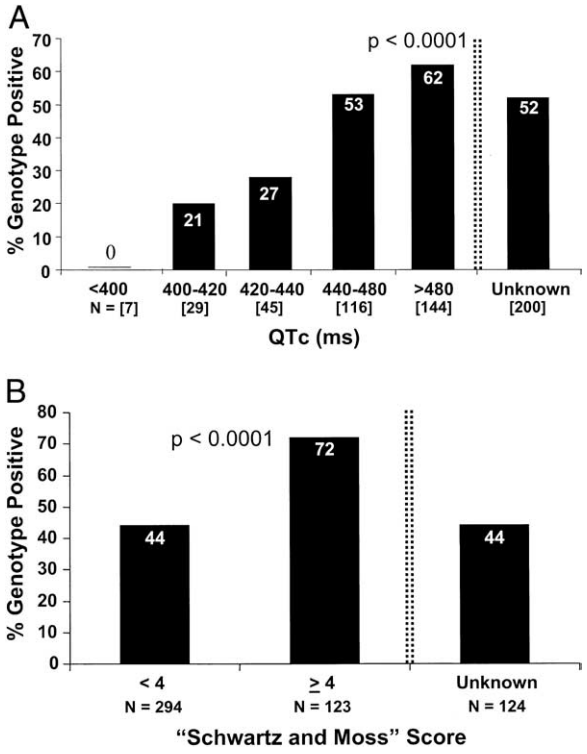

Figure 4. Effect of corrected QT interval (QTc) **(A)** and diagnostic score **(B)** on yield of genetic test. **(A)** The yield ranged from 0% when the subject's QTc was <400 ms to 62% when the QTc was >480 ms ($p < 0.0001$). **(B)** The greatest yield (72%) was achieved among the subset with a cumulative diagnostic score of ≥4, indicating strong clinical probability for long QT syndrome.

DISCUSSION

Surpassed only by the profound genetic heterogeneity that underlies LQTS, there is pronounced heterogeneity with respect to the yield of genetic testing that was impacted significantly and not surprisingly by the robustness of the clinical phenotype. Clearly, the genotype positive patients had greater QT intervals and cumulative diagnostic scores than those without an identifiable cardiac channel mutation. Among those having the highest clinical probability for LQTS, the yield was 72%.

Given the nonuniformity of clinical assessment by the nearly 200 different physicians represented in this study, this value (72%) may underestimate the true a priori likelihood of identifying an LQTS channel genotype in the next patient strongly suspected to have LQTS. For example, the

yield reached 83% in the subset of males diagnosed with LQTS during the first five years of life. In addition, following uniform clinical evaluation in our own LQTS clinic, the yield was 100% among those deemed to manifest a robust LQTS phenotype.

Nevertheless, based upon these data, cardiologists can expect the current genetic test to capture approximately three-fourths of LQTS. Thus, a negative genetic test in an index case with definite LQTS (i.e., genotype negative/phenotype positive LQTS) only informs the physician that the five channel genotypes have been excluded. Here, a negative genetic test provides no basis for removing the diagnosis and means that the physician will struggle with the correct classification of relatives (12,14).

In stark contrast, a positive genetic test may influence treatment decisions and will provide the means for precise "carrier" status classification of potentially at-risk relatives. With the increased recognition that a significant minority (25% to 50%) of individuals with genetically proven LQTS have a nondiagnostic QTc, the genetic test has become the new gold standard in the identification of concealed LQTS (18).

In contrast to its role among those with a definite LQTS phenotype, genetic testing may facilitate a move away from the diagnosis of LQTS even in light of its known 25% false negative rate. For example, many patients presently labeled with and treated for LQTS received the diagnosis based upon a clinical phenotype that would only support a low/intermediate clinical probability for the diagnosis. Equipped with an objective test (genetic testing) that effectively rules out 75% of LQTS, physicians may be more willing to consider reclassifying such low probability phenotype individuals as normal rather than persisting with the current default diagnosis of "borderline" LQTS (12).

Indeed, given the overall yield of 50%, this cohort almost certainly contains a spectrum of patients ranging from normal individuals with misdiagnosed vasovagal syncope to LQTS phenocopies such as catecholaminergic polymorphic ventricular tachycardia (CPVT) to correctly suspected LQTS. Among patients where there was an ECG for independent review and sufficient information was provided to assign a clinical score, nearly 60% had a QTc of $<$480 ms and over 70% had a clinical score of $<$4. Without genetic testing, this phenotypic presentation might translate into a diagnosis of "probable" or "borderline" LQTS, which typically results in initiation of medical therapy and restriction from competitive sports. Herein, over 40% of such subjects could be upgraded from a "probable/borderline" clinical assessment to a definitive genetic diagnosis, could be availed to genotype-guided management, and now have a definitive diagnostic biomarker available for their family, enabling precise preclinical presymptomatic classification of all relatives (12).

Undoubtedly, LQTS mimickers or phenocopies reside in this cohort. Among the subset of 81 patients referred for genetic testing but known to have a resting QTc of $<$440 ms, the yield of the LQTS genetic test was only 23%, indicating that the balance of this subset most likely represents nondisease status or perhaps the presence of an LQTS phenocopy such as CPVT (19). Previously, we demonstrated that 17 of these patients actually have CPVT (20,21). The clinical diagnosis in each patient was "atypical LQTS." We speculate that the referring physicians considered a clinical diagnosis of concealed LQT1 because of the near drowning, drowning, or exertional syncope phenotype in the setting of a nondiagnostic QTc. Thus, astute recognition of potential LQTS phenocopies such as CPVT and Andersen-Tawil syndrome (ATS1) will be essential to directing the patient's evaluation toward the proper molecular genetic test. Presently, *RyR2* (CPVT1), *KCNJ2* (ATS1), and *ANKB* (LQT4) mutation analyses are not included in the clinical LQTS genetic test (10).

Study limitations. It is intriguing to conjecture about the variable yields derived for the physicians who most used this research test. However, such speculations are made sparingly because of the major limitation associated with this study. In contrast to the rigid uniformity in genotyping this cohort, the phenotypic data were not procured similarly. Rather, we accepted a sample for genetic testing based solely upon either physician referral because of a tentative clinical diagnosis of LQTS or direct patient self-referral because of a physician-rendered diagnosis of LQTS.

Despite the voluntary submission of phenotypic data, there was excellent cooperation from the majority of referring physicians and self-referring patients and we were provided sufficient clinical and ECG information for the majority of cases. Further, the yield (44%) for the subset of patients with insufficient clinical information mirrored the yield of the subset whose clinical phenotype was "borderline." With this limitation as a caveat, we suspect that the referring physicians with yields $>$60% were primarily referring patients with a convincing phenotype for LQTS, whereas physicians associated with markedly lower yields were perhaps using this research test with a "rule-out" motive in mind. Alternatively, however, the extremely low yields may suggest that there is ongoing need for continuing medical education directed toward the proper clinical recognition of LQTS.

Conclusions. This study represents one of the largest series of consecutive unrelated patients referred for LQTS genetic testing. The identification of an LQTS channel genotype in one-half the cohort has permitted an in-depth analysis of the clinical parameters exerting the greatest impact on the yield of such genetic testing. In univariate analyses, ethnicity, age at diagnosis, QTc, "Schwartz and Moss" score, and referral source all impacted the yield of the genetic test. The observations described should assist in the proper utilization and diagnostic interpretation associated with LQTS genetic testing.

Acknowledgments

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REFERENCES

1. Ackerman MJ. The long QT syndrome: ion channel diseases of the heart. Mayo Clin Proc 1998;73:250–69.
2. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. Cell 2001;104:569–80.
3. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 1995;80:795–803.
4. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 1995;80: 805–11.
5. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation 2000;102:1178–85.
6. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. Heart Rhythm 2005;2:507–17.
7. Mohler PJ, Schott J-J, Gramolini AO, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature 2003;421:634–9.
8. Khositseth A, Ackerman MJ. Clinical evaluation, risk stratification, and management of congenital long QT syndrome. In: Gussak I, Antzelevitch C, editors. Contemporary Cardiology: Cardiac Repolarization: Bridging Basic and Clinical Science. Totowa, NJ: Humana Press, 2003:447–79.
9. Ackerman MJ. Cardiac channelopathies: it's in the genes. Nat Med 2004;10:463–4.
10. Genaissance Pharmaceuticals IPR. Genaissance Pharmaceuticals Launches its Proprietary Familion Test for Genetic Mutations Associated With Sudden Cardiac Death. PR Newswire, 2004.
11. Priori SG, Barhanin J, Hauer RN, et al. Genetic and molecular basis of cardiac arrhythmias; impact on clinical management. Study group on molecular basis of arrhythmias of the working group on arrhythmias of the European Society of Cardiology. Eur Heart J 1999;20:174–95.
12. Ackerman MJ. Genetic testing for risk stratification in hypertrophic cardiomyopathy and long QT syndrome: fact or fiction? Curr Opin Cardiol 2005;20:175–81.
13. Ackerman MJ. Cardiac causes of sudden unexpected death in children and their relationship to seizures and syncope: genetic testing for cardiac electropathies. Semin Pediatr Neurol 2005;12:52–8.
14. Shimizu W. The long QT syndrome: therapeutic implications of a genetic diagnosis. Cardiovasc Res 2005;67:347–56.
15. Ackerman MJ, Tester DJ, Jones G, Will MK, Burrow CR, Curran M. Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. Mayo Clin Proc 2003;78:1479–87.
16. Ackerman MJ, Splawski I, Makielski JC, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. Heart Rhythm 2004;1:600–7.
17. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome. An update. Circulation 1993;88: 782–4.
18. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. Circulation 1999;99:529–33.
19. Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 2001;103:196–200.
20. Choi G, Kopplin LJ, Tester DJ, Will ML, Haglund CM, Ackerman MJ. Spectrum and frequency of cardiac channel defects implicated in swimming-triggered arrhythmia syndromes. Circulation 2004; 110:2119–24.
21. Tester DJ, Kopplin LJ, Will ML, Ackerman MJ. Spectrum and prevalence of cardiac ryanodine receptor (RyR2) mutations in a cohort of unrelated patients referred explicitly for long QT syndrome genetic testing. Heart Rhythm 2005;2:1099–105.